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EDITORIAL

Do kidney tubules serve an angiogenic soup?

The podocyte is an in vivo source of vascular endothelial growth factor (VEGF), and VEGF-A maintains the morphologic integrity of the normal maturing glomerulus [1]. More controversial is whether renal tubules express VEGF, and whether, here, the factor has roles in health or disease. Do renal tubules express VEGF in vivo? VEGF-A can be immunodetected in proximal tubules and distal nephron, where protein levels and distribution can change markedly after acute tubular necrosis and subtotal nephrectomy [2–4]. In these contexts, VEGF-A protein levels correlate with peritubular capillary behavior, with either angiogenesis or capillary loss being observed, depending on the type of insult (e.g., toxic, ischemic, remnant kidney), the time after insult, and the experimental animal species (e.g., rat or mouse) and strain [2–4]. Kang et al [3] noted that, in several models of renal disease, the integrity of peritubular capillaries has a positive correlation with renal function; furthermore, in the rat remnant kidney, tubular VEGF-A becomes diminished, and the administration of VEGF-A minimizes loss of peritubular capillaries and stabilizes renal function [3]. Conversely, in mouse remnant kidneys, the progression of renal tubular lesions correlates with a robust angiogenic response by the peritubular microcirculation associated with an overall increase of renal VEGF-A protein [4].

The above data support a functional relationship between tubular VEGF-A and the state of the adjacent renal cortical microcirculation. A skeptic might, however, argue that the finding of immunoreactive VEGF-A in tubules simply reflects the fact that these epithelia take up the factor from either the tubule lumen or the microcirculation; in other words, the data do not prove that tubules make VEGF-A in vivo. On the other hand, there are several in situ hybridization studies that support the idea that renal tubules express VEGF-A transcripts in health, and that these levels can change after physiologic stress and in disease states. For example, Simon et al [5] reported that human fetal and adult kidney collecting ducts expressed VEGF transcripts, Marti and Risau [6] noted that adult mice expressed the factor in medullary ducts, and that here, VEGF was up-regulated with systemic hypoxia, and Yuan et al [2] found that proximal tubules of adult mice express VEGF-A transcripts and protein, and that both were down-regulated after folic acid–induced acute tubular necrosis.

Key words: angiogenesis, VEGF, TGFβ, tubule, Smad, kidney.

To further define factors that modulate the expression of VEGF-A by renal tubules, investigators have turned to studies of cultured cells. Kidney tubule cells express VEGF-A when grown in vitro, and levels of mRNA and protein can be up-regulated by hypoxia and other stimuli, such as transforming growth factor β1, and down-regulated by interleukins [3, 7, 8]. Binding of active TGFβ to cell surface receptor serine/threonine kinases leads to a series of biochemical reactions whereby the signal is transduced to the cell nucleus; this involves the direct phosphorylation of receptor-mediated Smad 1, 2, 3, 5, and 8, intracellular proteins which are expressed in most cells [9]. The expression of Smad 6 and 7 proteins are more tightly regulated, and these inhibitory Smads are induced by TGFβ in an autoregulatory feedback system [9].

In a study reported in this issue of *Kidney International*, Nakagawa et al [10] show that the up-regulation of VEGF-A expression by rat proximal tubule cells exposed to TGFβ1 is linked to the Smad system, and the authors have additionally begun to explore the complexity of TGFβ1 actions with regard to the responses of other molecules, which themselves modify angiogenesis. These include: (*1*) the soluble form of Flt-1 (also called VEGF receptor type 1), a molecule which binds to VEGF-A but which does not transduce an angiogenic response; Flt-1, therefore, acts as a natural antagonist of VEGF-A [11]; and (*2*) thrombospondin-1 (TSP-1), a molecule which activates latent TGF-β1, and which is itself anti-angiogenic [4]. Nakagawa et al [10] found that TGFβ1 increased expression of both VEGF-A and TSP-1 mRNA and protein in proximal tubule cells, and also increased Smad 2 and 3 phophorylation/activation. Furthermore, forced overexpression of Smad7 inhibited all of these TGFβ1-induced effects. Using cells from mice which were null-mutant for specific Smad genes, the authors went on to demonstrate that Smad3 was essential for the VEGF-A response, while TSP-1 and soluble Flt-1 stimulation required Smad2; a potential criticism of this part of the study is that fibroblasts, rather than tubular cells, were used. In the last part of the study, the authors investigated the effects of conditioned media on proliferation of human umbilical venous endothelial cells as a surrogate marker of "angiogenesis." Media from TGF1β-stimulated Smad2 null-mutant fibroblasts enhanced proliferation, while media conditioned by Smad3 null-mutant fibroblasts failed to do so. This was consistent with above observations that the balance of expressed angiogenic/antiangiogenic factors pre-

sumably differs between wild-type, Smad2, and Smad3 mutant cells; similar experiments were not, however, reported with null-mutant proximal tubule cells.

So, do kidney tubules serve an angiogenic soup? As outlined above, there is plenty of evidence that they express VEGF-A in vivo, where levels correlate with expansion or regression of peritubular capillaries. Final proof for the postulated functional roles of tubule-derived VEGF-A in health and disease will require the generation and study of genetically engineered mice in which VEGF-A is ablated, specifically in parts of the renal tubule (i.e., an inducible Cre-lox system) whereby VEGF-A is deleted in proximal tubules or collecting ducts, perhaps using γ-glutamyl transpepidase and aquaporin 2 promoters to drive Cre recombinase expression. Similar strategies, with VEGF-A ablated in podocytes, were informative for understanding glomerular biology [1]. In addition, the work of Nakagawa et al [10] alerts us to the fact that kidney tubule cells in vitro, and therefore, probably in vivo, also express a range of antiangiogenic factors; hence, the biological response of an endothelial cells near a tubule will depend on the balance of epithelial-derived stimulatory and inhibitory factors. The fact that specific intracellular molecules (e.g., Smad2 and 3) affect this balance suggests a possibility that endothelial responses could be therapeutically shifted towards angiogenesis (e.g., in states such as kidney tubular atrophy associated with capillary loss), or antiangiogenesis (e.g., in renal cancers where vessels maintain tumor viability), if the activation of specific Smad proteins could be chemically modulated.

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